

Pulmonary Hypertension Caused by Congestive Heart Failure Is Ameliorated by Long-Term Application of an Endothelin Receptor Antagonist

Increased Expression of Endothelin-1 Messenger Ribonucleic Acid and Endothelin-1-Like Immunoreactivity in the Lung in Congestive Heart Failure in Rats

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Objectives. The purpose of this study was to investigate whether 1) endothelin-1, a potent vasoconstrictor peptide, is involved in progression of pulmonary hypertension caused by congestive heart failure (CHF); and 2) whether long-term treatment with BQ-123, an endothelin receptor antagonist, ameliorates pulmonary hypertension caused by CHF.

Background. Congestive heart failure accompanies pulmonary hypertension, and the severity of pulmonary hypertension is an important determinant of prognosis. Although we reported that production of endothelin-1 is increased in the failing heart in rats with CHF, it is not known whether production of endothelin-1 in the lung is altered by CHF.

Methods. Congestive heart failure was induced by coronary artery ligation in rats. Expression of preproendothelin-1 messenger ribonucleic acid (mRNA) in the lung and kidney was determined. Endothelin-1 staining (immunoreactivity) in the lung was studied by immunohistochemical analysis. Effects of long-term BQ-123 treatment on the rats were studied.

Results. Two weeks postoperatively, CHF accompanied by pulmonary hypertension developed in the rats (CHF rats). Expression of preproendothelin-1 mRNA in the lung was markedly higher in the CHF rats than in the sham-operated rats, whereas that in the kidney did not differ between the two groups. Endothelin-1 staining on the pulmonary vascular endothelial cells was more intense in the CHF rats. BQ-123 treatment over a 2-week period in the CHF rats greatly reduced right ventricular systolic pressure and central venous pressure, but it did not affect blood pressure or left ventricular contractility (peak positive first derivative of left ventricular pressure) in these rats.

Conclusions. Long-term BQ-123 treatment greatly ameliorated pulmonary hypertension in the CHF rats. The present study suggests that endothelin-1 plays an important role in the progression of pulmonary hypertension caused by CHF and that an endothelin receptor antagonist may be a new therapeutic agent for CHF-induced pulmonary hypertension.

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The severity of congestive heart failure (CHF) often correlates with the degree of elevation of pulmonary artery pressure (1). Various heart and lung diseases are often accompanied by pulmonary hypertension, and the severity of this pulmonary hypertension is an important determinant of the prognosis of these patients (2). Although the exact factors that promote pulmonary hypertension in these diseases are unknown, it is

thought that there are some differences in mechanisms for progression of pulmonary hypertension among patients with heart and lung diseases (2).

Endothelin-1, a potent vasoconstrictor peptide produced by vascular endothelial cells (3-6), possesses several properties that promote the development of pulmonary hypertension. First, endothelin-1 contracts isolated pulmonary vessels (6-8) and increases pulmonary vascular resistance (6,9). Second, it has a mitogenic effect on vascular smooth muscle cells (5,6,10) and fibroblasts (6,11) that is consistent with a role in vascular remodeling, a prominent finding in pulmonary hypertensive stages. In rats with monocrotaline-induced pulmonary hypertension, a model of pulmonary hypertension caused by inflammatory lung disease, we (8) reported that long-term treatment with an endothelin receptor antagonist significantly inhibited the progression of pulmonary hypertension and prevented the thickening of the vascular walls of the pulmonary artery. These

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Abbreviations and Acronyms

cDNA	=	complementary deoxyribonucleic acid
CHF	=	congestive heart failure
CHF rat	=	rat with surgically induced congestive heart failure
ECG	=	electrocardiographic
ETA	=	endothelin-A
ETB	=	endothelin-B
mRNA	=	messenger ribonucleic acid
+dP/dt _{max}	=	peak positive first derivative of left ventricular pressure

findings suggested that endogenous endothelin-1 is involved in the progression of pulmonary hypertension caused by inflammatory lung disease. However, it is not known whether endothelin-1 is involved in the progression of pulmonary hypertension caused by CHF or whether long-term treatment with an endothelin receptor antagonist is effective in ameliorating pulmonary hypertension caused by CHF.

Plasma levels of endothelin-1 were reported to be increased in patients (12-14) and experimental animals (15,16) with CHF. In rats with CHF, we (16) reported that the heart is one of the origins of elevated plasma endothelin-1, because both messenger ribonucleic acid (mRNA) levels and mature peptide levels are markedly increased in the failing hearts of these rats. However, it is unclear whether the production of endothelin-1 in the lungs is altered by CHF and it is not known whether long-term treatment with an endothelin receptor antagonist is effective in ameliorating pulmonary hypertension caused by CHF. Cody et al. (12) reported that plasma endothelin-1 concentration correlates with the extent of pulmonary hypertension in patients with CHF, suggesting that elevated circulating endothelin-1 is related to the change in lung conditions in these patients. However, because the lung is an important organ for eliminating endothelin-1 from the circulation (17), it is not known whether this phenomenon is caused by increased production of endothelin-1 in the lungs or decreased elimination of endothelin-1 by the lungs. Thus, the measurement of plasma endothelin-1 levels has limitations for assessing production of endothelin-1 in the lung, and there is no report that directly indicates whether or not endothelin-1 production in the lung is increased in CHF. A study of the expression of preproendothelin-1 mRNA in the lung would provide an answer to this question, as would a study of the distribution of endothelin-1 staining in the lung section.

The goal of this study was to investigate whether endothelin-1 is involved in the progression of pulmonary hypertension caused by CHF in rats and whether long-term treatment with an endothelin receptor antagonist ameliorates pulmonary hypertension in these rats. For this purpose, we investigated the expression of preproendothelin-1 mRNA in the lung of rats with CHF and the effect of long-term administration of BQ-123, an endothelin-A receptor (ETA receptor) antagonist (18), on pulmonary hypertension in rats with CHF induced by coronary artery ligation. Furthermore, we

investigated the distribution of endothelin-1 staining in the lung section by immunohistochemical methods using antiendothelin-1 antibody.

Methods

Experimental heart failure. The left coronary artery was ligated in the rat to serve as a model of CHF. This rat (CHF rat) is a well established model (19,20) in which pathophysiologic changes are similar to those seen in ischemic heart disease, that is, the most common contemporary cause of heart failure in humans (21). Left ventricular free wall myocardial infarction was induced in male Sprague-Dawley rats (weighing 170 to 200 g) according to the method of Pfeffer et al. (19), as we (16) have previously described in detail. In brief, each rat was anesthetized with ether. A thoracotomy was performed, the heart was rapidly exteriorized and the proximal portion of the left coronary artery was ligated with a 5-0 silk suture. Except for coronary artery ligation, sham-operated rats underwent an identical procedure. About 60% of the rats with myocardial infarction died within the 1st 24 h, the surviving rats were maintained on standard rat chow and water ad libitum for 2 weeks. The study was approved by University of Tsukuba and conformed to the "Position of the American Heart Association on Research Animal Use" adopted by the Association in November 1984.

Seven days postoperatively, myocardial infarction was confirmed by electrocardiography; for these experiments we used only rats having abnormal Q waves in both limb leads I and aVL, as these findings have been reported (20) to represent a medium to large infarct size. In a previous study we (16) found that under these conditions, and with electrocardiographic (ECG) assessment congestive heart failure develops in the rats within a few weeks after operation...

Hemodynamic measurement and tissue sampling. On the day of the experiment, the rats were anesthetized with sodium pentobarbital (50 mg/kg body weight intraperitoneally). A microtip pressure transducer catheter (model SPC-320, Millar Instruments) was inserted into the right carotid artery. After arterial blood pressure was measured, the catheter was advanced into the left ventricle for evaluation of left ventricular pressure. These hemodynamic measurements were recorded by a polygraph system (AP-601G amplifier and WT-687G thermal pen recorder, Nihon Kohden, Tokyo, Japan). Moreover, the peak positive first derivative of left ventricular pressure (left ventricular +dP/dt_{max}) was derived by active analog differentiation of the pressure signal differentiation amplifier (ED-601G, Nihon Kohden). Subsequently, a polyethylene catheter was inserted into the right jugular vein to measure central venous pressure and right ventricular pressure. We (8,16) have previously described details of the methods for measuring these hemodynamic variables. After hemodynamic measurement a blood sample was collected from the right carotid artery. The lung and kidney were excised, weighed and frozen in liquid nitrogen. The heart was weighed after it was divided into the right and left ventri-

cles (including the septum) under cold Krebs-Ringer solution. The plasma and tissue samples were stored at -80°C for mature endothelin-1 peptide assay and for determination of preproendothelin-1 mRNA expression. Some of these rats were used for immunohistochemical study.

Sandwich-enzyme immunoassay to determine plasma endothelin-1 levels. Plasma endothelin-1 concentration was measured by a sandwich-enzyme immunoassay as we (8,16,22) have previously described. In brief, each blood sample was placed in a chilled tube containing aprotinin (300 kallikrein-inhibiting U/ml) and EDTA (2 mg/ml) and was centrifuged at 3,000 g for 15 min at 4°C . Sandwich-enzyme immunoassay for endothelin-1 was carried out as previously described by using immobilized mouse monoclonal antibody AwETN40, which recognizes the N-terminal portion of endothelin-1, and peroxidase-labeled rabbit antiendothelin-1 C-terminal peptide (15-21) Fab' (8,16,22). The assay for endothelin-1 did not cross-react with endothelin-3 or big-endothelin-1 (cross-reactivity $<0.1\%$) (8,16,22).

Northern blot analysis for preproendothelin-1 mRNA in the lung and kidney. Total RNA was prepared from the lung and kidney of sham-operated rats and the CHF rats, by selective precipitation in 3-mol/liter lithium chloride and 6-mol/liter urea as we (8,16,23) have previously described. In brief, total RNA (10 $\mu\text{g}/\text{lane}$) from the lung and the kidney was separated by formamide/1.1% agarose gel electrophoresis and transferred onto nylon membranes (Hybond N, Amersham, Buckinghamshire, United Kingdom). The membranes were hybridized with a phosphorus-32-labeled complementary deoxyribonucleic acid (cDNA) probe. After hybridization, the filter was finally washed in $0.1 \times$ standard saline citrate/0.1% sodium dodecyl sulfate at 50°C and autoradiographed with intensifying screens at -80°C for 24 h. The preproendothelin-1 cDNA used as a probe in the present study was a previously described full-length insert of lambda r-endothelin-1-2 (8,16,23) that was labeled by random priming with [α -phosphorus-32] deoxycytidine triphosphate (dCTP) ($\sim 3,000$ Ci/mmol, Amersham). To normalize the preproendothelin-1 signals for the loaded amounts and transfer efficiencies, the same membranes were rehybridized with beta-actin cDNA probe or ethidium bromide staining of 18S ribosomal RNA as the internal control.

Immunohistochemical analysis for endothelin-1 staining in the lungs. Tissue endothelin-1 staining (endothelin-1-like immunoreactivity) was studied by using methods we (4,24) have previously described with minor modifications. In brief, after hemodynamic measurement, the lungs were subjected to perfusion fixation with 0.01 mol/liter periodate-0.075 mol/liter lysin-2% paraformaldehyde solution, and fixed in this solution at 4°C overnight. The specimens were embedded in paraffin wax (melting point 58°C). The sections were cut into 3- μm thickness and stained immunohistochemically by using the biotin-streptavidin-horseradish peroxidase method for the detection of endothelin-1. Rabbit antiendothelin-1 polyclonal antibody (Peninsula Lab., Inc.) was used as the primary antibody. Deparaffinized sections were incubated with rabbit

antiendothelin-1 antibody (dilution 1:400) at room temperature for 3 h. After being washed, the sections were incubated with secondary antibody diluted at 1:100 (biotinylated antirabbit immunoglobulin; IBL, Fujioka, Japan) for 30 min, then with horseradish peroxidase-conjugated streptavidin diluted at 1:100 for 30 min. Immunoreactive endothelin-1 products were visualized with 0.05% 3,3'-diamino-benzidine-0.02% hydrogen peroxide. The slides were counterstained with methyl green. The specificity of the immunoreactivity was determined by an absorption test; that is, the consecutive sections were subjected to the same procedure with the use of the supernatant of antiendothelin-1 antibody (dilution 1:100) preabsorbed with synthetic endothelin-1 (20 $\mu\text{mol}/\text{liter}$) at 4°C for overnight.

Effects of continuous BQ-123 infusion for 2 weeks on hemodynamic variables in the CHF rats. To investigate the effects of continuous BQ-123 infusion on the CHF rats, an osmotic minipump (model 2ML2, Alza Co.) was subcutaneously implanted in the CHF rats and sham-operated rats on the day after myocardial infarction. BQ-123, donated by Dr. Masaru Nishikibe (Tsukuba Research Institute, Banyu Pharmaceutical Co., Tsukuba, Japan), was dissolved in saline solution. The osmotic minipumps contained either saline solution alone or saline solution and BQ-123. The rats were separated into three groups as follows: group 1, CHF rats receiving continuous BQ-123 infusion (15 mg/rat per day) for 2 weeks; group 2, CHF rats receiving continuous saline infusion for 2 weeks; and group 3, sham-operated rats receiving continuous saline infusion for 2 weeks.

Two weeks after the osmotic minipump was implanted, it was extracted under ether anesthesia 24 h before the experiments; that is, the hemodynamic measurements were performed after a 24-h washout period for BQ-123. The hemodynamic variables were measured under pentobarbital anesthesia (50 mg/kg body weight).

Infarct size was measured as previously described (16,19) in both the CHF rats treated with saline solution and the CHF rats treated with BQ-123. In brief, after being weighed, the heart was immersion-fixed in 10% buffered formalin and then cut into four transverse sections from apex to base. These sections were processed in standard fashion and embedded in paraffin. A Masson trichrome-stained thin section from each level was projected, and the perimeters of the infarcted and noninfarcted epicardial and endocardial surfaces were traced and digitized. The infarcted portion (the proportion of the infarcted left ventricle) was calculated from these measurements (16,19).

Statistical analysis. All statistical comparisons were performed by using a statistical package for the Macintosh personal computer (STAT VIEW, version 4.0, Abacus Concepts, Inc.). Differences between data for the CHF and sham-operated rats were assessed by an unpaired *t* test. One-way analysis of variance followed by Bonferroni's multiple comparison test was used for statistical comparison among the three treatment groups (see Table 2, Fig. 4). Differences were considered significant at $p < 0.05$.

Table 1. Hemodynamic Variables in Sham-Operated Rats and CHF Rats 2 Weeks Postoperatively

Group	HR (beats/min)	mBP (mm Hg)	LVSP (mm Hg)	LV + dP/dt _{max} (mm Hg/s)	LVEDP (mm Hg)	RVSP (mm Hg)	RVEDP (mm Hg)	CVP (mm Hg)
Sham-operated rats (n = 6)	378 ± 14	112 ± 6	128 ± 5	8,696 ± 452	3.6 ± 0.7	28.8 ± 1.0	1.9 ± 0.2	1.8 ± 0.3
CHF rats (n = 6)	357 ± 7	89 ± 3	99 ± 4	5,648 ± 335	19.3 ± 2.0	46.0 ± 2.8	10.6 ± 1.2	3.1 ± 0.4
p value	NS	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.05

Values presented are mean value ± SEM. CHF = congestive heart failure; CVP = central venous pressure; HR = heart rate; LVEDP = left ventricular end-diastolic pressure; LV + dP/dt_{max} = peak positive first derivative of left ventricular pressure; LVSP = left ventricular systolic pressure; mBP = mean blood pressure; RVEDP = right ventricular end-diastolic pressure; RVSP = right ventricular systolic pressure.

Results

Hemodynamic variables and tissue weights. Mean arterial blood pressure, left ventricular peak systolic pressure and left ventricular + dP/dt_{max} were significantly lower in the CHF rats than in the sham-operated rats (Table 1). Left ventricular end-diastolic pressure was markedly elevated in the CHF rats (Table 1), and right ventricular systolic, right ventricular end-diastolic and central venous pressures were significantly higher in the CHF rats than in the sham-operated rats (Table 1). These data indicate that CHF accompanied by pulmonary hypertension developed in the CHF rats.

The CHF rats weighed less than the sham-operated rats (277 ± 8 vs. 307 ± 4 g, $n = 6$, $p < 0.05$), and their lung weight mass index for body weight was much higher (8.53 ± 0.63 vs. 3.90 ± 0.14 mg/g, $p < 0.01$), suggesting that they had pulmonary congestion. Right ventricular mass index for body weight was significantly higher in the CHF rats than in the sham-operated rats (1.17 ± 0.09 vs. 0.60 ± 0.02 mg/g, $p < 0.01$), but left ventricular mass index for body weight was not different in the two groups (2.31 ± 0.08 vs. 2.17 ± 0.06 mg/g, $p = NS$).

Plasma endothelin-1 level. The plasma endothelin-1 concentration of the CHF rats was ~3.3-fold higher than that of the sham-operated rats (3.89 ± 0.49 vs. 1.32 ± 0.10 pg/ml, $n = 6$, $p < 0.01$).

Expression of preproendothelin-1 mRNA in the lung and kidney. The expression of preproendothelin-1 mRNA in the lung of both the CHF rats and the sham-operated rats was determined by Northern blot analysis. Typical examples of the lung at 2 weeks after operation are shown in Figure 1. The expression of preproendothelin-1 mRNA in the lung in the CHF rats was much higher than that in the sham-operated rats (Fig. 1). Densitometric analysis of these blots corrected for the levels of beta-actin mRNA, which was used as normalization for a constitutively expressed message in the lung. The densitometric ratio of (preproendothelin-1 mRNA)/(beta-actin mRNA) in the lung of the CHF rats was significantly higher than that in the sham-operated rats (0.38 ± 0.04 vs. 0.15 ± 0.02 , $n = 5$, $p < 0.01$). However, in the kidney, the expression of preproendothelin-1 mRNA did not differ between the two rat groups (Fig. 2).

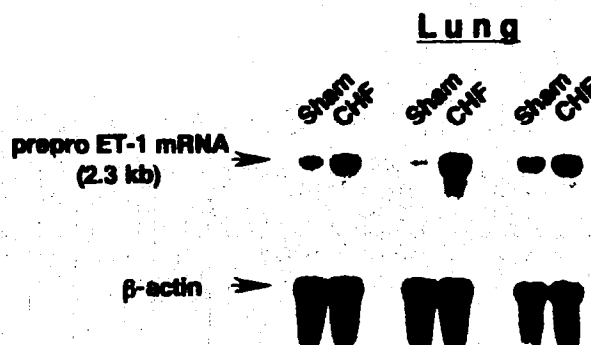
Immunohistochemical analysis for endothelin-1 staining in the lungs. The typical examples of endothelin-1 staining (i.e., endothelin-1-like immunoreactivity) in the rat lung demonstrated by immunohistochemical methods are shown in Figure

3. The endothelin-1 staining (endothelin-1-like immunoreactivity) appeared brown (Fig. 3). The vascular endothelium was stained brown, indicating that endothelin-1 is distributed in the vascular endothelium of both the sham-operated rats (Fig. 3, left) and the CHF rats (Fig. 3, right). Figure 3 shows that the intensity of the endothelin-1 staining (endothelin-1-like immunoreactivity) on the vascular endothelial cells of the pulmonary artery was more intense in the CHF rats than in the sham-operated rats. Results similar to those shown in Figure 3 were observed in six CHF rats and six sham-operated rats. However, the intensity of the endothelin-1 staining on the alveolar macrophages did not differ between the two rat groups (data not shown).

The absorption test showed that the endothelin-1-like immunoreactivity was greatly reduced when the primary antibody was absorbed with synthetic endothelin-1 ($20 \mu\text{mol/liter}$) (data not shown), indicating that the immunoreactivity was specific for endothelin-1.

Effects of continuous BQ-123 infusion on hemodynamic variables and tissue weights. Continuous infusion of BQ-123 ($15 \text{ mg/rat per day}$) for 2 weeks caused significant reductions in right ventricular systolic pressure (by 48%, Fig. 4, left), right ventricular end-diastolic pressure (by 49%, Table 2) and central venous pressure (by 75%, Fig. 4, right) in the CHF rats.

Figure 1. Typical examples of Northern blot analysis are shown for the level of preproendothelin-1 (prepro ET-1) messenger ribonucleic acid (mRNA) in the lung of the sham-operated rats (Sham) and the rats with congestive heart failure (CHF) 2 weeks after operation. To normalize the preproendothelin-1 signals for the loaded amounts and transfer efficiencies, beta (β)-actin mRNA was considered and is indicated by arrows. The expression of preproendothelin-1 mRNA in the lung of the CHF rats was greatly increased. kb = kilobase.



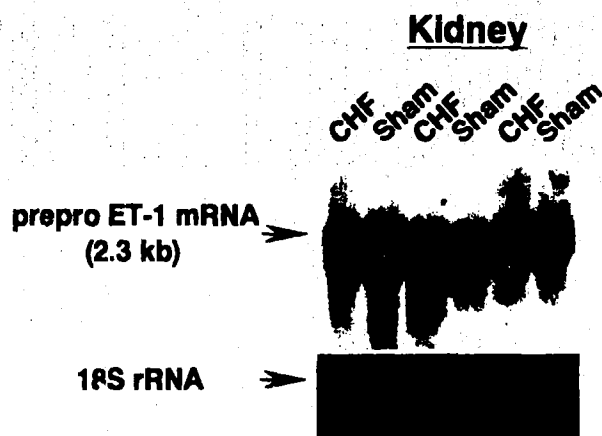


Figure 2. Typical examples of Northern blot analysis are shown for the level of preproendothelin-1 (prepro ET-1) messenger ribonucleic acid (mRNA) in the kidney of the sham-operated rats (Sham) and the rats with congestive heart failure (CHF) 2 weeks after operation. To normalize the preproendothelin-1 signals for the loaded amounts and transfer efficiencies, ethidium bromide staining of 18S ribosomal ribonucleic acid (rRNA) was considered and is indicated by arrows. The expression of preproendothelin-1 mRNA in the kidney of CHF rats did not differ from that of sham-operated rats. kb = kilobase.

Thus, continuous infusion of BQ-123 for 2 weeks greatly ameliorated pulmonary hypertension in the CHF rats. It did not affect mean blood pressure, left ventricular $+dP/dt_{max}$ or left ventricular end-diastolic pressure in this group (Table 2). However, because we did not measure cardiac output or blood flow in the aorta, we cannot know how BQ-123 treatment affected the afterload of the left ventricle.

Figure 3. Typical examples of endothelin-1 staining (endothelin-1-like immunoreactivity) in the rat lung demonstrated by the immunohistochemical method in a sham-operated rat (A) and a rat with chronic heart failure (CHF) (B) 2 weeks after operation. The endothelin-1 staining (endothelin-1-like immunoreactivity) was observed as brown (arrows). The vascular endothelium was stained brown, indicating that endothelin-1 is distributed in the vascular endothelium of both rats. The intensity of the endothelin-1 staining (endothelin-1-like immunoreactivity) on the vascular endothelial cells of the pulmonary artery was stronger in the CHF rats than in the sham-operated rats. Results similar to those shown here were observed in six CHF rats and six sham-operated rats.

The ratio of the weight of the right ventricle to body weight was significantly higher in the CHF rats treated with saline solution than in the sham-operated rats treated with saline solution (1.26 ± 0.06 vs. 0.60 ± 0.02 mg/g, $n = 6$, $p < 0.01$). Long-term BQ-123 treatment significantly ($p < 0.05$) ameliorated right ventricular hypertrophy. The ratio of the weight of the left ventricle to body weight did not differ among three groups; that is, the ratios in the sham-operated rats treated with saline solution, the CHF rats treated with saline solution and the CHF rats treated with BQ-123 were 2.20 ± 0.04 , 2.33 ± 0.06 and 2.36 ± 0.09 (each $n = 6$), respectively.

The mean myocardial infarct size in the CHF rats treated with saline solution ($49 \pm 4\%$) was not different from that in the CHF rats treated with BQ-123 ($50 \pm 5\%$).

Discussion

Effects of long-term treatment with an endothelin receptor antagonist on pulmonary hypertension caused by CHF. At 2 weeks after coronary artery ligation, congestive heart failure accompanied by pulmonary hypertension developed in the rats. The expression of preproendothelin-1 mRNA was markedly increased in the lung of the CHF rats and endothelin-1 staining in the vascular endothelial cells of the pulmonary artery was more intense in the CHF rats than in the sham-operated rats. Furthermore, the present study demonstrated for the first time that long-term treatment with BQ-123, an endothelin-A receptor (ET_A receptor) antagonist (18), greatly ameliorated pulmonary hypertension in the CHF rats, suggesting that an endothelin receptor antagonist may be a potential therapeutic agent for pulmonary hypertension caused by CHF. The latter finding is very important because pulmonary hypertension caused by CHF is resistant to many drugs and is often a progressive condition that ultimately leads to right heart failure and death (2). The present findings suggest that endogenous endothelin-1 greatly contributes to the progression of pulmonary hypertension caused by CHF in rats. It has been reported that endothelin-1-induced vasoconstriction of the pulmonary artery is mainly mediated by endothelin ET_A receptors in rats (6,25,26) and humans (27).

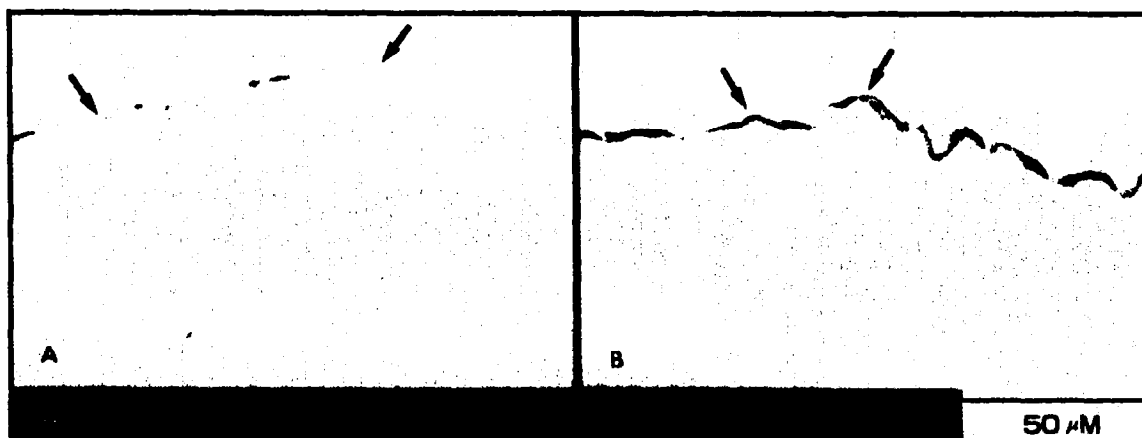
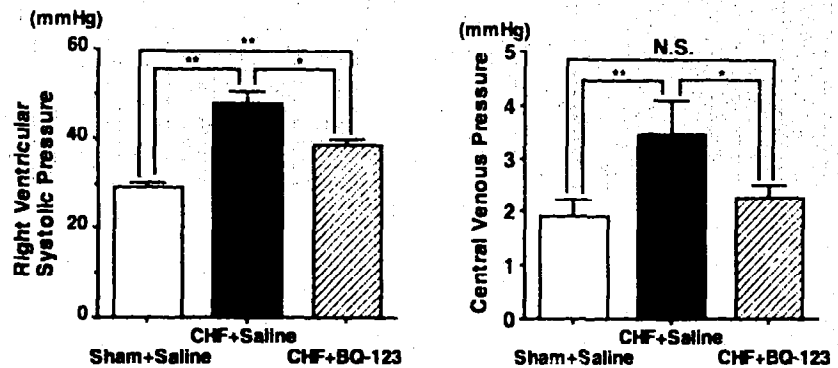


Figure 4. Bar graph shows the effects of the continuous infusion of BQ-123, an endothelin-A receptor antagonist on the progression of pulmonary hypertension (left panel) and systemic congestion (right panel) in the rats with congestive heart failure (CHF). Sham = sham-operated rats. * $p < 0.05$; ** $p < 0.01$.



Production of endothelin-1 in the pulmonary vascular endothelial cells in the CHF rats. Cody et al. (12) reported that plasma endothelin-1 concentration correlates with the extent of pulmonary hypertension in patients with CHF, suggesting that elevated circulating endothelin-1 is related to the change in lung conditions in these patients. However, controversy persists regarding the production of endothelin-1 in the lung in CHF. Tsutamoto et al. (28) showed that the plasma endothelin-1 level was increased significantly in plasma from the main pulmonary artery to that in the pulmonary capillary wedge region in patients with CHF, whereas Eaton et al. (29) reported the absence of pulmonary endothelin gradient in patients with normal or elevated circulating endothelin. Because the lung is an important organ for eliminating endothelin-1 from the circulation (17), the measurement of plasma endothelin-1 levels has limited ability to assess production of endothelin-1 in the lung. In the present study, our findings are novel in that we have directly demonstrated that the production of endothelin-1 in the lung is increased in CHF, because the expression of preproendothelin-1 mRNA in the lung was greatly increased in the CHF rats. As the expression of preproendothelin-1 mRNA in the kidney did not differ between the CHF rats and the sham-operated rats, the increase in endothelin-1 production is a relatively specific phenomenon in the lung of the CHF rats. The present immunohistochemical study also revealed that endothelin-1 staining in

the vascular endothelial cells of the pulmonary artery was more intense in the CHF rats than in the sham-operated rats. Therefore, it is suggested the increased peptide level of endothelin-1 in the pulmonary vascular endothelial cells in the CHF rats is attributed to the increase in the endothelin-1 gene transcription.

The reason for the enhanced expression of preproendothelin-1 mRNA in the lung of CHF rats remains to be elucidated. It has been reported that the expression of preproendothelin-1 mRNA was increased by hemodynamic shear stress in cultured vascular endothelial cells (30) and that pure pressure without shear stress or stretch enhanced the release of endothelin-1 in cultured human endothelial cells (31). These results suggest that production of endothelin-1 in vascular endothelial cells may be increased by mechanical factors. Therefore, it can be assumed that hemodynamic overload to the pulmonary vascular endothelium by pulmonary hypertension causes the increase in the expression of preproendothelin-1 mRNA in the pulmonary vascular endothelium in CHF rats. This argument is in agreement with our finding (32) that elevated levels of circulating plasma endothelin-1 in young patients with pulmonary hypertension caused by congenital heart disease were decreased in accordance with amelioration of pulmonary hypertension by successful surgical repair.

Expression of endothelin-1 in pulmonary hypertension due to different etiologies. Plasma endothelin-1 concentration was reported to be elevated in animal models of pulmonary hypertension caused by lung diseases (i.e., rats with pulmonary hypertension induced by hypoxia [33] or monocrotaline [8]). In rats with monocrotaline-induced pulmonary hypertension, a rat model of pulmonary hypertension caused by inflammatory lung disease, we (34) reported that the intensity of endothelin-1 staining in the pulmonary artery was same as that in control rats, whereas the intensity of endothelin-1 staining in the alveolar macrophages was markedly increased. In patients with pulmonary hypertension caused by noninflammatory lung disease or plexogenic pulmonary arteriopathy, Giaid et al. (35) reported that the expression of endothelin-1 is increased in the pulmonary vascular endothelial cells.

Selective effects of BQ-123 on the pulmonary circuit to lower arterial pressure without affecting the passive component of pulmonary hypertension in CHF. In this study, BQ-123 affected neither left ventricular $+dP/dt_{max}$ nor left ventric-

Table 2. Hemodynamic Variables of Sham-Operated Rats and CHF Rats Given BQ-123 or Saline Infusion for 2 Weeks

Group	mBP (mm Hg)	LV + dP/dt _{max} (mm Hg/s)	LVEDP (mm Hg)	RVEDP (mm Hg)
Sham + saline (n = 6)	116 ± 4	9,040 ± 176	3.4 ± 0.7	1.9 ± 0.3
CHF + saline (n = 6)	86 ± 3*	5,520 ± 278*	21.7 ± 1.4*	11.3 ± 0.8*
CHF + BQ-123 (n = 6)	82 ± 3*	5,467 ± 313*	21.2 ± 1.1*	6.7 ± 0.6*†

* $p < 0.01$ versus the corresponding value for Sham + saline rats. † $p < 0.01$ versus the corresponding value for CHF + saline rats. Values presented are mean value ± SEM. CHF + BQ-123 = CHF rats given continuous BQ-123 infusion for 2 weeks; CHF + saline = CHF rats given continuous saline infusion for 2 weeks; Sham + saline = sham-operated rats given continuous saline infusion for 2 weeks. Other abbreviations as in Table 1.

ular end-diastolic pressure, indicating that it did not affect the impaired function of the left ventricle in the CHF rats. Thus, the administration of BQ-123 for 2 weeks did not affect the condition of left heart failure in rats with CHF. It has been considered that the pulmonary hypertension seen in isolated left heart failure is due to predominantly passive phenomena caused by elevated left-sided pressures (1). Nevertheless, the present study showed that long-term BQ-123 treatment greatly reduced pulmonary artery pressure but not systemic arterial pressure in the CHF rats. Thus, BQ-123 selectively reduced pulmonary artery pressure in the CHF rats. One possible explanation for this finding is that there is a major vasoactive component to pulmonary hypertension seen in left heart failure and endothelin-1 may be a candidate for the component.

It has been reported that endothelin-1-induced vasoconstriction in various rat arteries that participate in the regulation of the systemic circulation is mainly mediated by ETA receptors (25) and that BQ-123 greatly antagonizes the pressor effect of exogenously applied endothelin-1 in rats (16,18,36). Therefore, we consider that the difference between the effects of BQ-123 on pulmonary artery pressure and on systemic pressure may be attributed to the difference in the production of endothelin-1 in the vascular endothelium between the pulmonary and systemic circulations in the CHF rats. This consideration is partly supported by the finding that the expression of preproendothelin-1 mRNA in the CHF rats is increased in the lung but not in the kidney.

Our previous study (16) showed that short-term administration of the ETA receptor antagonist BQ-123 did not alter mean blood pressure in the CHF rats. However, Teerlink et al. (37) reported that short-term administration of the ETA/endothelin-B (ET_B) combined receptor antagonist bosentan lowers blood pressure in rats with CHF due to myocardial infarction. One possible explanation for the discrepancy in short-term effects on systemic blood pressure between BQ-123 and bosentan is that the blockade of both ETA and ET_B receptors by bosentan may contribute to its blood pressure-lowering effect in CHF rats, because both ETA-mediated and ET_B-mediated mechanisms for vascular contraction exist in various vessels (6,25). In the present study, long-term BQ-123 treatment did not affect mean arterial pressure in the CHF rats. However, it is not known whether long-term treatment with ETA/B combined receptor antagonists affects systemic blood pressure of the CHF rats. In patients with severe chronic heart failure (New York Heart Association class III), Kiowski et al. (38) reported that short-term (2-h) infusion of the ETA/B combined receptor antagonist bosentan significantly reduced mean systemic arterial pressure by 7.7% and pulmonary artery pressure by 13.7%. However, there is no report whether long-term treatment (at least 2 to 3 weeks) with endothelin antagonists alters systemic arterial pressure or pulmonary artery pressure in patients with chronic heart failure.

Effects of endothelin receptor antagonists on myocardial infarct size. This heart failure model—that is, rats with myocardial infarction—is well established, and some investigators have reported the effects of endothelin antagonists on this

model. Controversial findings were reported in several studies concerning the effects of endothelin antagonists on myocardial infarct size. Watanabe et al. reported that a monoclonal antibody against endothelin-1 (39) or the ETA/B receptor antagonist TAK-044 (40) reduced myocardial infarct size in this model, and Lee et al. (41) also reported that the ETA receptor antagonist FR-139317 reduced myocardial infarct size in this model. However, in a rabbit model of acute myocardial ischemia and reperfusion, McMurdo et al. (42) observed that FR-139317 had no effect on infarct size. In the present study, the ETA receptor antagonist BQ-123 had no effect on the infarct size, in accordance with the study's finding that long-term (2 weeks) BQ-123 treatment did not affect left ventricular $+dP/dt_{\max}$ or left ventricular end-diastolic pressure in the CHF rats. The reason for these differences among studies is unclear.

Study limitations. In the present study, treatment with BQ-123 was started on the day after infarction and continued for 2 weeks. This time period was selected for assessment of the long-term effects of BQ-123 on the pulmonary hypertension caused by CHF, because we (16) have previously found in this model that at this stage CHF-induced pulmonary hypertension is marked but myocardial hypertrophy of the surviving myocardium in the left ventricle is less marked. At later stages (several months after infarction), we observed (unpublished data) that significant myocardial hypertrophy occurs in this animal. In the present study, BQ-123 treatment over a 2-week period did not affect the impaired function of the left ventricle in the CHF rats despite significant reductions in right ventricular systolic pressure and central venous pressure. Thus, BQ-123 greatly ameliorated right heart failure in the CHF rats without ameliorating the condition of left heart failure in these rats at this stage. However, because an endothelin receptor antagonist has been reported to prevent cardiac hypertrophy *in vitro* (6,43) and *in vivo* (6,8,44), it remains to be assessed whether longer treatment periods (e.g., several months) would ameliorate left ventricular remodeling (hypertrophy of cardiac myocytes in the surviving myocardium, cavity enlargement of the left ventricle, among others) or the left heart failure in the CHF rats.

In the present study, we did not investigate whether long-term BQ-123 treatment affects the preproendothelin-1 mRNA expression or endothelin-1-like immunoreactivity in the lung of CHF rats. Therefore, it is still unclear whether long-term BQ-123 treatment affects the regulation of endothelin-1 production in the lung of CHF rats.

Our previous report (8) indicated that marked thickening of the vascular walls of the pulmonary artery occurred in the rats with pulmonary hypertension caused by inflammatory lung disease due to monocrotaline and that long-term treatment with BQ-123 greatly inhibited this thickening. However, the present study showed slight thickening of the vascular walls of the pulmonary artery in the CHF rats (Fig. 3); therefore, it is difficult to assess whether long-term BQ-123 treatment inhibits the thickening of the vascular walls of the pulmonary artery in CHF rats. The CHF rats used in the present study were studied 2 weeks postoperatively; however, we have found (unpublished observation) that the marked thickening of the vascular walls

of the pulmonary artery occurs in the CHF rats 3 months postoperatively. Therefore, additional study using the CHF rats 3 months postoperatively is required to resolve this question.

In the present study, right ventricular systolic pressure of the sham-operated rats appeared to be relatively higher than that in humans. Because we measured the right ventricular pressure with a fluid-filled system at high heart rates (~350 beats/min), we consider that the time constant was not sufficient to allow an adequate response, and this may be a reason for the relatively high pressure.

Conclusions. The expression of preproendothelin-1 mRNA was greatly increased in the lung of the CHF rats and the endothelin-1 staining in the vascular endothelial cells of the pulmonary artery was more intense in the CHF rats than in the sham-operated rats. Furthermore, continuous infusion of BQ-123 for 2 weeks greatly ameliorated pulmonary hypertension in the CHF rats. Therefore, the present study suggests that endogenous endothelin-1 contributes to the progression of pulmonary hypertension caused by CHF in rats and that an endothelin receptor antagonist may be a new therapeutic agent for pulmonary hypertension caused by CHF.

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